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Embryology of *Wisteria floribunda* (Willd.) DC. (Leguminosae) and the Taxonomic Position of the Genus

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フジの胚学的研究およびフジ属の分類学的位置

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Wisteria floribunda (Willd.) DC. was studied embryologically. The anther is tetrasporangiate; its wall development corresponds to the dicotyledonous type; the tapetum is of the glandular type and uninucleate; the pollen tetrad is tetrahedral; pollen grains are 2-celled at the time of shedding. The ovule is campylotropous, bitegmic and crassinucellate; the archesporium is multicellular and the megaspore tetrad is T-shaped; chalazal spore is functional and following development of the embryo sac is of the Polygonum type; the synergids and antipodals are ephemeral; the endosperm development is of the Nuclear type.

It is different from other members of the tribe Millettieae in such embryological features as the pollen cell numbers, the female archesporium numbers, the megaspore tetrad pattern and shape of the embryo sac. These results support the taxonomic position of *Wisteria* being excluded from the Millettieae proposed on the basis of structural differences of chloroplast DNA between *Wisteria* and other members of the tribe.

Introduction

Prakash (1987) reviewed embryological studies of Leguminosae and noticed that only 35 of 650 genera in the family have been studied in detail. These genera were generally studied on sporogenesis, gametogenesis and embryogeny including the endosperm development. The genus *Wisteria* of the tribe Millettieae in the subfamily Papilionoideae is not included in the 35 genera. On the embryology of the genus, only the male sporogenesis of *W. floribunda* reported by Roscoe (1927) and the female sporogenesis and gametogenesis of *W. sinensis* studied by Rembert (1967, 1971) are known.

The purpose of this study is to investigate the features of embryology of *Wisteria floribunda*, especially the anther formation and the endosperm development, because these features have not been studied in any species of the genus. Results are compared with those of the previous knowledges on *Wisteria* and other representatives of Millettieae, i.e., *Pongamia* (Anantaswamy Rau 1951), *Millettia* (Pal 1960) and *Tephrosia* (Anantaswamy Rau 1951; Johri and Garg 1959; Ashrafunnisa and Pullaiah 1989; Seshavatharam 1982).

Materials and methods

Various stages of flowers and fruits had been collected from wild plants of *Wisteria floribunda* (Willd.) DC. growing spontaneously in the campus of Tohoku University in Aobayama, Sendai, from March to October in 1986 and 1987. Voucher specimens are kept in TUS. After fixation with FAA and dehydration by ethanol-buthanol series, the samples were embedded in paraplast and sectioned at 10 micrometers in thickness. Staining was performed with safranin and fast green.

Observations

Anther, microsporogenesis and male gametophyte. The anther is tetrasporangiate (Figs. 1E, E'). The archesporium is hypodermal and differentiated into a primary parietal layer towards outside and a primary sporogenous layer towards inside. At the stage when pre-sporogenous cells become discernible in size, the primary parietal layer undergoes periclinal division forming two secondary parietal layers between the epidermis and a mass of pre-sporogenous cells (Fig. 1A). The inner parietal layer develops directly into the tapetum, while the outer layer divides once again forming the endothecium towards outside and the middle layer towards inside (Figs. 1B, C). The middle layer partially divides into two layers (Fig. 1B). According to Davis (1966), this pattern of the anther wall development is of the dicotyledonous type.

In the mature anther, the epidermis persists as a flattened layer (Fig. 1D) and the endothecium develops fibrous thickenings (Figs. 1D, E, E'). The middle layers get crushed and degenerated during meiosis (Figs. 1C, D). The tapetum is rich at the pollen tetrad stage. It is of the glandular type and each cell is uninucleate (Fig. 1C).

The primary sporogenous cells undergo anti- and periclinal divisions forming a mass of pollen mother cells (Figs. 1B, F) which are discernible from the parietal cells in their larger size. Being enveloped by callose membrane, the pollen mother cells undergo meiotic division to form 4-nucleate cells (Figs. 1G–I). Following cytokinesis is simultaneous and tetrahedral pollen tetrads are formed (Figs. 1C, J). The microspore just after separation is distorted and develops exine (Fig. 1K).

Pollen grains at the time of shedding are 2-celled (Fig. 1L) with three germ pores and smooth and thick exine.

Ovary, ovule, megasporogenesis and female gametophyte.

The ovary is monocarpellary, uniloculate with about 10 ovules on the marginal placentae (Fig. 2A).

The ovule is bitegmic and crassinucellate (Fig. 2G). Integuments are originated by anticlinal division of epidermal cells (Fig. 2D). At maturity, the micropyle is formed by both integuments as a zig-zag path, and the ovule is campylotropous (Fig. 2H).

The female archesporium is hypodermal and multicellular (Fig. 2B).

The archesporial cells undergo periclinal division giving parietal cells towards outside and a megaspore mother cell towards inside. Then the megaspore mother cell becomes larger in size (Fig. 2C).

The megaspore mother cell undergoes meiosis resulting in a T-shaped megaspore tetrad (Fig. 2E). The chalazal megaspore is functional while the micropylar three megaspores degenerate (Fig. 2F). The functional megaspore elongates and undergoes nuclear divisions resulting in an 8-nucleate, 7-celled embryo sac which is of the Polygonum type (Figs. 3A–E). The shape of the embryo sac is ellipsoid.

Two polar nuclei fuse at the center of the embryo sac forming a diploid secondary nucleus before fertilization (Fig. 3F).

The synergids and the antipodal cells are ephemeral.

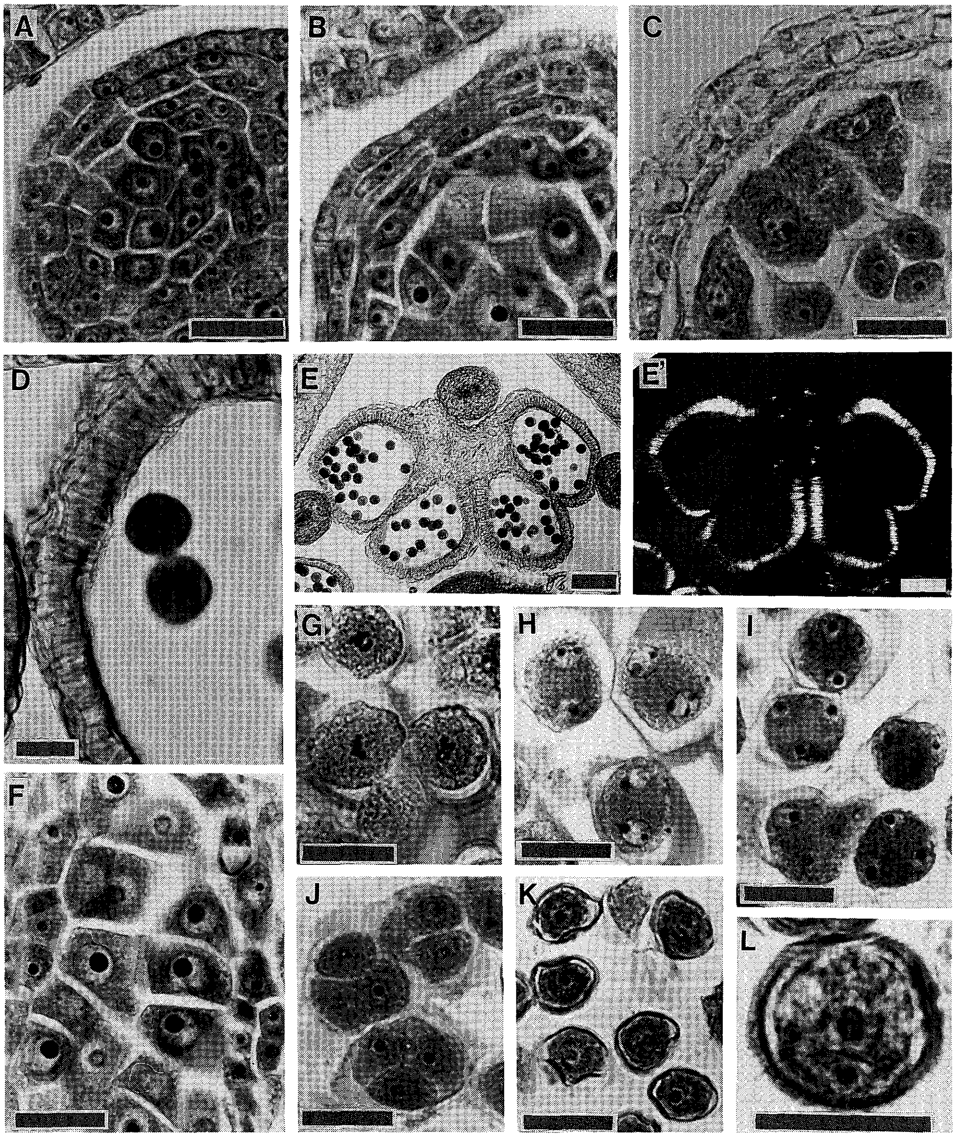

Fig. 1. Anther, microsporogenesis and male gametophyte of *Wisteria floribunda*. A. Loculus of anther at pre-sporogenous stage. B. Anther wall at pollen mother cell stage. C. Anther wall at pollen tetrad stage. D. Anther wall at maturity. E. Cross-sectioned anther with four loculi. E'. The same scene under polarlized light. Fibrous tissue detectable. F. Pollen mother cells. G. Miosis at the beginning. H. Interkinesis. I. Telophase. J. Tetrahedral pollen tetrads enveloped by callose membrane. K. Pollen grains just after tetrads. L. Mature pollen grain. Bar A–D and F–L 20 μm; E, E' 100 μm.

Fertilization and endosperm

Fertilization is porogamous (Fig. 4A). The zygote divides transversely resulting in a terminal cell (ca) and a basal cell (cb) (Fig. 4B). Each cell divides vertically to form a decussate proembryo (Fig. 4C). The terminal cells provide the most part of the embryo, while the basal cells undergo less divisions and become larger in size to form a suspensor (Figs. 4D, E).

The endosperm nuclear divisions result in large

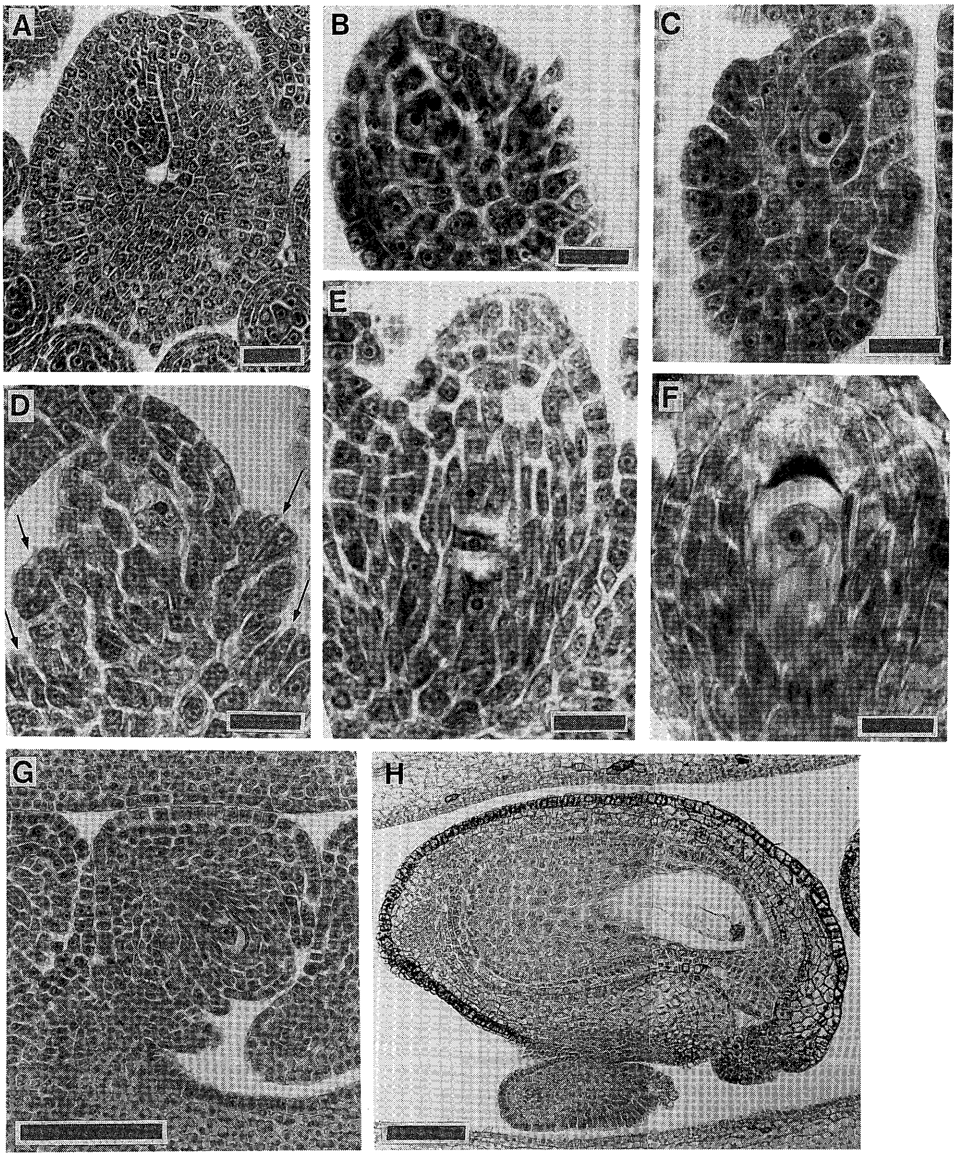

Fig. 2. Ovary, ovule and Megasporogenesis of *Wisteria floribunda*. A. Cross-sectioned carpel with initiating placenta. B. Multicellular female archesporium. C. Megaspore mother cell. D. Initiative outer and inner integuments (arrows). E. T-shaped megaspore tetrad. F. Functional chalazal megaspore. G. Ovule just before miosis. H. Mature campylotropous ovule with a micropyle. Bar A 40 μm; B–F 20 μm; G–H 100 μm.

number of free nuclei which are distributed in the periphery (Fig. 4F). At about the globular stage of the embryo the micropylar part of the endosperm becomes cellular (Fig. 4G). This type of endosperm development is of the Nuclear type.

Discussions

Three-celled pollen at the time of shedding and formation of the unicellular female archesporium are reported in *Tephrosia* (Seshavatharam 1982; Ashrafunnisa and Pullaiah 1989) and *Millettia* (Pal 1960). These features may be important to define

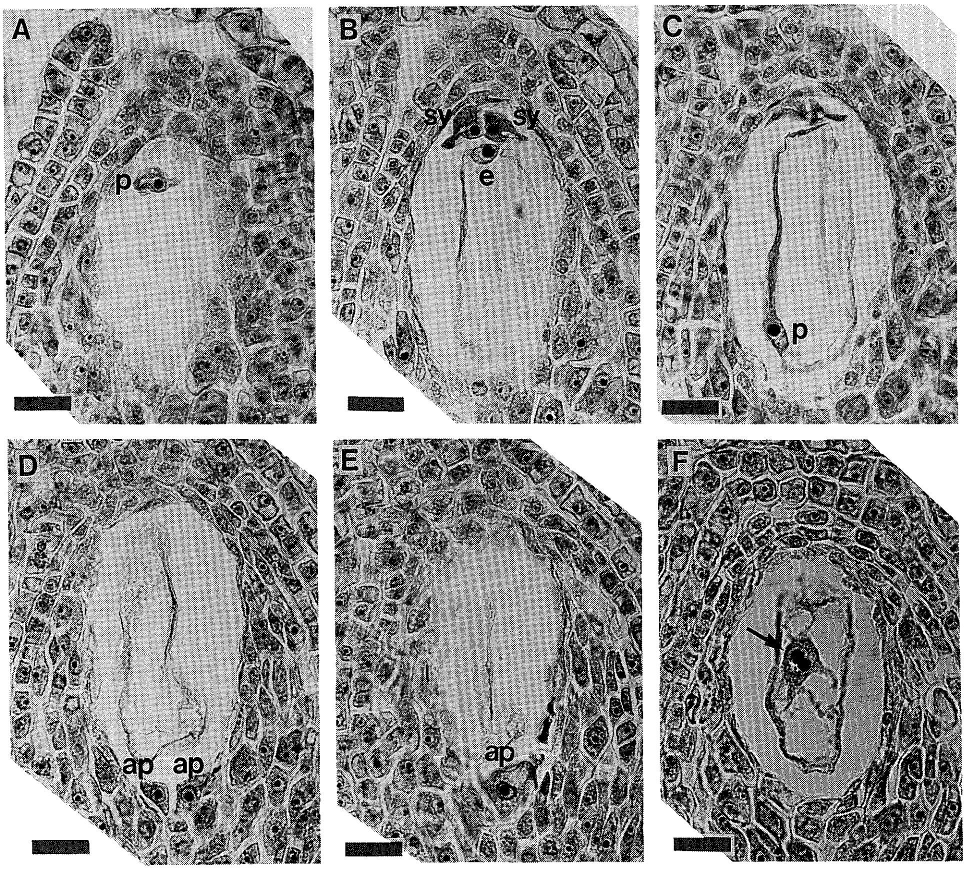

Fig. 3. Embryo sac of *Wisteria floribunda*. A. One of polar nuclei. B. Two synergids and an egg cell at the micropylal end. C. Another pollar nucleus. D. Two antipodals at chalazal end. E. Another antipodal at chalazal end. F. Polar nuclei (arrow) fusing before fertilization. A–E are from the same embryo sac. ap: antipodal. e: egg cell. p: polar nucleus. sy: synergid. Bar A–F 20 μm.

Millettieae in the embryological characteristics. Although there is very little information on the embryology of the tribe Millettieae, the pollen grains are 2-celled at the time of shedding and the female archesporium is multicellular in the majority of Leguminosae, especially in the subfamily Papilionoideae (Prakash 1987). In *Wisteria*, however, the 2-celled pollen at the time of shedding and the multicellular female archesporium are observed.

The linear megaspore tetrad is reported in *Millettia* (Pal 1960) and *Tephrosia* (Seshavatharam 1982; Ashrafunnisa and Pullaiah 1989), and the ovate embryo sac at maturity in *Millettia* (Pal 1960), and the oval embryo sac in *Tephrosia* (Ashrafunnisa and Pullaiah 1989). But in *Wisteria* the megaspore tetrad is T-shaped with a functional chalazal megaspore and the mature embryo sac is ellipsoid. These embryological characters of the megaspore tetrad pattern and shape of the mature embryo sac have been known in Leguminosae without any relations to the systematic position of taxa having these differences. The endosperm development was studied in *Pongamia* (Anantaswamy Rau 1951), *Millettia* (Pal 1960) and *Tephrosia* (Sesha-vatharam 1982; Ashrafunnisa and Pullaiah 1989). These features are common to *Wisteria*, and also to the majority of other Leguminosae

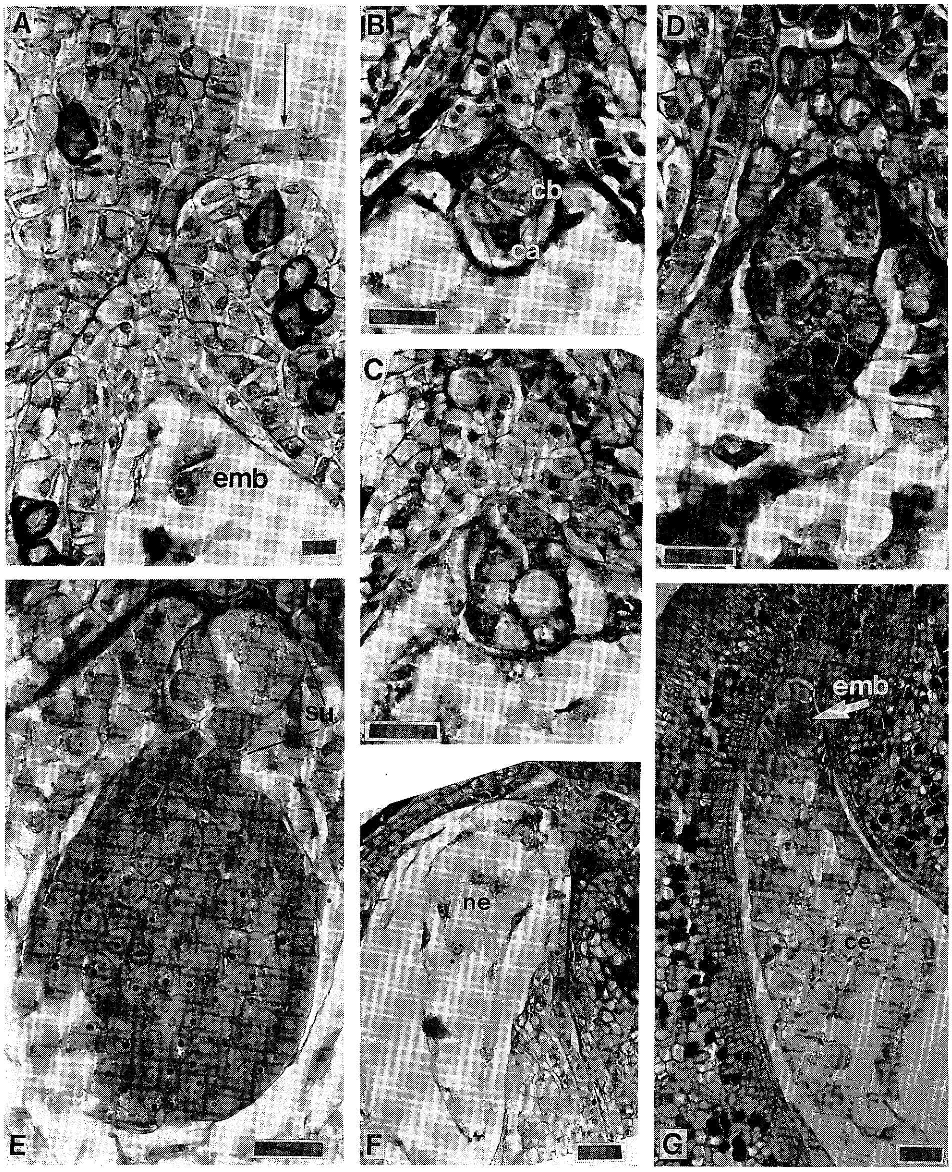

Fig. 4. Fertilization, embryogenesis and endosperm of *Wisteria floribunda*. A. Pollen tube (arrow) after porogamous fertilization. B. 2-celled pro-embryo. C. Decussate pro-embryo. D. Cell wall formation in endosperm initiating around embryo. E. Developing embryo with suspensor. F. Endosperm with free nuclei just after fertilization. G. Cellular part of endosperm with globular embryo. ca: terminal cell. cb: basal cell. ce: cellular part of endosperm. emb: embryo. ne: free-nuclear endosperm. su: suspensor. Bar A–E 25 μm; F, G 100 μm.

with known embryological features. The differences in embryological specificities between *Wisteria* and other representatives of the tribe Millettieae are summarized in Table 1.

The taxonomic position of *Wisteria* has been discussed by Geesink (1981, 1984), Lavin (1987), Palmer et al. (1988), Lavin et al. (1990) and Doyle et al. (1992). Especially recently the genus has been treated

Table 1. Comparative characters in the embryology of the genera of Millettieae, Galegeae and the majority of Leguminosae

	Pollen cell number	Female archesporium	Megaspore tetrad
Wisteria (present study)	2	multicellular	T-shaped
Tephrosia (Seshavatharam 1982; Ashrafunnisa and Pullaiah 1989)	3	unicellular	linear
Millettia (Pal 1960)	3	unicellular	linear
Astragalus (James 1950)	2	unicellular	linear
Majority of Leguminosa (Prakash 1987)	2	multicellular	linear / T-shaped

in molecular systematics. According to Palmer et al. (1988), *Wisteria* is the only member of Millettieae that lacks the inverted repeat of chloroplast DNA. The inverted repeat is present in most legumes, but is absent in such groups of temperate herbaceous tribes of Papilionoideae as Galegeae, Vicieae, Cicereae, etc. Palmer et al. (1988) suggested a close relation of *Wisteria* to the tribe Galegeae based on additional characters common to them in the distribution pattern and chromosome number. Doyle et al. (1992 on page 234) stated that "the placement of *Wisteria* as a derived number of Millettieae, but basal to the temperate herbaceous group as suggested by the loss of the inverted repeat, is not inconsistent with morphologic data."

James (1950) showed an unicellular female archesporium and a linear tetrad of the megaspore on the genus *Astragalus*, a member of the tribe Galegeae. These features show clear embryological differences between *Wisteria* and *Astragalus*, and seem to suggest an inconsistency between *Wisteria* into Galegeae. However, evidences from the embryology on these taxa are extremely scanty for consideration of relationships between them.

On the other hand, Rembert (1971) discussed phylogenetic relations of the tribes of Leguminosae based on patterns of megasporogenesis, i.e. types of the female tetrad. According to him, the patterns of *Wisteria sinensis* are the loss of cell wall between two micropylar spores in T-shaped tetrad and between chalazal two in linear tetrad. The former pattern is also observed in *Vicia*, and the latter in *Lathyrus*, respectively. These genera are included in the tribe Vicieae, which also lack the inverted repeat of chloroplast DNA (Palmer et al 1988). However, *Wisteria floribunda* has T-shaped megaspore tetrad. This pattern is, according to Rembert (1971), classified into different pattern from that of *W. sinensis*. The pattern of *W. floribunda* occurs widely among the Leguminosae (Rembert 1971). *Wisteria* and *Vicia* are common in having the multicellular female archesporium and the 2-celled pollen grains at the time of shedding (Mitchell 1975), but linear megaspore tetrad is observed in *Vicia* (Martin 1914; Mitchell 1975). Much more informations on the embryology of Vicieae are required to consider the relationships between *Wisteria* and Vicieae.

Our result supports the previous suggestions proposed by Lavin (1987), Palmer et al. (1988) and Doyle et al. (1992) based on molecular characters that the taxonomic position of *Wisteria* in the tribe Millettieae is anomalous, but gives no evidences for the relation between *Wisteria* and Galegeae or Vicieae.

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要旨

フジの胚学的研究を行った．フジの属するナツフジ連の胚学的研究はシナフジ（**Rembert 1967, 1971**）のほかに，*Tephrosia* 属（**Seshavatharam 1982, Ashrafunnisa and Pullaiah. 1989**），ナツフジ属（**Pal 1960**），クロヨナ属（**Anantaswamy Rau 1951**）について行われてきたが，このうち *Tephrosia* 属とナツフジ属を除く植物については雌性胞子・配偶子形成や胚乳形成に限った部分的な研究しかなされていなかった．本研究はフジについて雄性・雌性の胞子形成および配偶子形成，さらに胚発生と胚乳形成を調べ，上記，ナツフジ連の種類の胚学的特徴と比較することを目的とした．フジの胚学的特徴は以下の通りであった．

葯室は4，葯壁形成は双子葉型，タペータムは分泌型で単核性，小胞子四分子は四面体型で成熟した花粉は2細胞であった．胚珠は彎生胚珠，珠皮は2，厚層珠心で雌性胞原細胞は多細胞，大胞子四分子はT型であり，胚嚢形成はタデ型，胚嚢の形状は長円形であった．受精は珠孔受精，胚乳は遊離核型であった．

これらのうち花粉の細胞数，雌性胞原細胞の数および大胞子四分子の型で，フジはナツフジ連の他の属と異なる特徴を示した．なかでも花粉の細胞数が3であり，雌性胞原細胞が単細胞であることがマメ科におけるナツフジ連の胚学的特異性であり，この点における不一致はフジのナツフジ連への帰属を考える上で重要である．フジの従来の分類学的位置は近年，おもに葉緑体 **DNA** の形質から疑問視され（**Palmer et al. 1988**），むしろゲンゲ連に近いという見解を示している．しかし，胚学的特徴からはフジをゲンゲ連に結び付ける共通性は見いだされない．